Human Vaccines & Immunotherapeutics 9:8, 1661–1672; August 2013; © 2013 Landes Bioscience

The potential of 1018 ISS adjuvant in hepatitis B vaccines

HEPLISAV™ review

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Keywords: hepatitis B, vaccine, adjuvants, immune responses, 1018 ISS, immunostimulatory sequences, seroprotection, alum

Hepatitis B (HBV) virus infects the liver, and upon chronic infection, can cause liver cirrhosis and hepatocellular carcinoma. Despite universal vaccination programs against the virus, HBV still affects over 2 billion people worldwide, with over 240 million developing a chronic infection. While current alum-adjuvanted vaccines have shown efficacy in promoting seroprotection in healthy adults, 5–10% of immune-competent populations fail to achieve long-lasting seroprotection from these formulations. Furthermore, a large proportion of immunocompromised patients fail to achieve seroprotective antibody titers after receiving these vaccines. A novel vaccine candidate, HEPLISAV™, uses immunostimulatory sequences (ISS), in its formulation that helps induce a robust humoral and cell mediated immunity against HBV. In Phase III clinical trials, HEPLISAV™ has been shown to elicit seroprotective antibody titers with fewer immunizations. Similar safety profiles are demonstrated when compared with current HBV vaccines. For these reasons, HEPLISAV™ is an attractive vaccine to combat this global disease.

Introduction

The hepatitis B virus (HBV) causes a potentially life-threatening liver infection that affects over two billion people worldwide, with over 240 million being chronically infected and unable to clear the virus.[1] It is also estimated that more than 600,000 will die annually from hepatitis B-infection-related complications. As such, this disease remains a major global health concern, and carries a higher risk of liver-related death than hepatitis C.[2]

HBV is 50–100 times more infectious than HIV, can be transmitted by contact with blood or other bodily fluids and can survive outside of the body for at least 7 d. Modes of HBV transmission include perinatal (from mother to baby), sexual contact, sharing of needles and blood transfusions. Geographically, HBV is endemic in China and other parts of Asia, where 8–10% of the adult population is chronically infected; less than 1% of chronic cases occur in Western Europe and North America. On average, the incubation period of HBV is 3 mo; however, HBV can be detected within 1–2 mo after infection.[1] Symptoms during acute infections include jaundice, dark urine, extreme fatigue, nausea, vomiting and abdominal pain; however, most people are asymptomatic. Chronic infections often develop into liver cirrhosis and/or hepatocellular carcinoma. Morbidity and mortality as a result of chronic HBV infections are significant. Surgery and chemotherapy may help to prolong life after early diagnosis, however in developing countries due to lack of proper medical care most people die within months of infection.[1]

HBV, a member of the *Hepadnaviridae* family, is a 42 nm virion that replicates in humans and other higher primates, but is unable to replicate in vitro.[3] The virus comprises a nucleocapsid and an outer envelope of hepatitis B surface antigen (HBsAg), which self-assembles into nanoparticles that form and expose a highly immunogenic "a" epitope determinant, the basis of HBV vaccines on the market. The nucleocapsid contains hepatitis B core antigen (HBcAg), a DNA polymerase-reverse transcriptase, viral genome of 3.2 kb, and other cellular proteins.[4,5] HBcAg undergoes post-translational modification to become hepatitis B "e" antigen (HBeAg), which is a marker for high viral replication and infectivity.[5] Finally, the hepatitis B "x" antigen (HBxAg) is principally involved in the development of liver cancer by upregulating hepatocellular growth and survival genes and blocking TNF-α-mediated killing of the infected cells.[6]

Studies have shown that host HBV-specific T cell responses are important in determining the progression of, or recovery from infection.[7] Indeed, viral clearance in the liver correlated with upregulated T cell-derived IFN-γ, demonstrating the importance of adaptive T-cell responses in inhibiting viral replication and killing infected cells.[8] This is also demonstrated in acute HBV infections where broad polyclonal cytotoxic T lymphocyte (CTL) responses persist after clearance.[9-12] Studies in chimpanzees showed that CD8 cell depletion led to prolonged infection and delayed HBV clearance; only when CD8 cells were returned to baseline levels did HBV-specific responses occur, including increased IFN-γ and viral clearance.[13] Unfortunately, CTL responses in chronic HBV infections are generally weak.[7] It is unclear whether T cell deletion, exhaustion, anergy or dysfunction contributes to poor T-cell responses.[14] Future studies will be important to understand this phenomenon.

Since their inception in the 1980s, HBV vaccines have generally fared very well in terms of inducing protective immune responses according to the recommended immunization schedule

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Submitted: 02/20/13; Revised: 04/08/13; Accepted: 04/16/13
http://dx.doi.org/10.4161/hv.24715

in healthy adolescents and adults. However, their impact on controlling the global incidence rates was minimal, not due to vaccine ineffectiveness, but to the populations to which the vaccines were targeted. Initially, this population was the "high-risk" group, which includes health care workers and hyporesponsive populations such as hemodialysis patients and the immunocompromised; however, less than half of the hepatitis cases occurred in the high-risk groups.[15] Additionally, concerns arose since first generation HBV vaccines were derived from the plasma of asymptomatic viral carriers, which carried the possibility of disease transmission.[16] As such, recombinant vaccine antigens were derived from yeast, which led to the development of the currently marketed HBV vaccines, such as Engerix-B® and Recombivax HB®. The safety profiles of these vaccines are not in doubt with more than 25 y of available data.

Furthering the success of the HBV vaccines, the World Health Assembly passed a resolution in 1992 to recommend universal hepatitis B vaccination. This led to an increase in the number of countries that have HBV vaccination programs from 31 to 179 (as of July 2011).[1] Worldwide HBV vaccination programs have proven to be effective in preventing mother to infant transmission, chronic infections, and decreased incidence of hepatocellular carcinoma.[17] HBV vaccines also do not interfere with the immune responses from other vaccines and vice versa.[3] This is particularly important since infants receive many vaccinations early in life. Since unimmunized infants born to hepatitis B infected mothers are 3.5 times more likely to become infected with HBV, WHO recommended that newborns should be immunized against HBV within 24 h after birth.[18] From 2006–2008, newborn HBV vaccination increased from 27% to 69% worldwide.[3] In Taiwan, where the world's first HBV universal vaccination program began in 1984, the prevalence rate dropped from 9.8% to 1.3% ten years later in children under 15 y of age.[19] With recent reductions in the price of HBV vaccines, vaccination programs are becoming even more widespread in developing countries, and facilitate the reduction of HBV-related complications.

All licensed vaccines are comprised of HBsAg because of its effect on B and T-cell responses. Its persistence in chronic infections is the principal marker for the risk of developing long-term liver disease and hepatocellular carcinoma.[3] The "a" determinant from HBsAg is also very immunogenic. Current HBV vaccines available in North America and Europe are formulated with recombinant HBsAg adsorbed to aluminum hydroxide or aluminum phosphate adjuvant. Some of the newer vaccines entering the market also use monophosphoryl lipid (MPL) as an adjuvant. Long-term seroprotection against HBV requires HBsAg antibody (anti-HBs) titers to be greater than or equal to 10 mIU/ml 1–3 mo after final immunization based on studies that examined plasma-derived and recombinant HBV vaccines.[15,20,21] At this concentration, protection is conferred in 95% of children and young adults after completion of the vaccine regimen.[22,23] Once the vaccination series is completed, a number of studies have showed that HBsAg-carrier status or clinical HBV rarely occurs, even when the concentration of anti-HBs becomes less than 10 mIU/ml over time, stressing the importance of completing the vaccination program.[24-26]

Typically, seroprotection is achieved in a three dose series at 0, 1, and 6 mo post-initial intramuscular administration of the vaccine, although factors such as age, obesity, smoking, diabetes and renal disease can result in lower rates of protection.[27] Other regimens include an accelerated schedule for hyporesponsive individuals[28] and two-dose administrations for those who may not desire the typical three-dose series or in developing countries where compliance may be difficult to achieve.[29] Despite current vaccination programs, failure rates to achieve seroprotective anti-HBs titers are still high among individuals over 40 (25–50%)[30] and those who undergo hemodialysis or have diabetes (30–40%).[31] In addition, the 6-mo schedule of 3 injections can often be difficult to comply for developing countries or for individuals who need rapid seroprotection, such as health care workers. As such, addressing vaccine hyporesponsiveness, the inability to maintain seroprotective anti-HBs titers and reducing the number of immunizations are important considerations for HBV vaccines.

In this article, we will review current prophylactic licensed HBV vaccines in terms of efficacy (immune responses, seroprotection) and safety. In particular, HEPLISAV™, a Dynavax product, will be compared with commercially available vaccines, as a potential HBV vaccine for licensure. With mounting challenges of hyporesponsive populations, more rapid seroprotection in high-risk groups such as health care workers, and increased compliance with reduced costs in HBV-endemic countries, the need for effective HBV prophylaxis is paramount. Vaccination is cost-effective, for all ages, regardless of prevalence, when compared with the health costs associated with HBV-related complications.[32,33]

Key Issues

Hepatitis B virus (HBV) infection is a potentially life-threatening liver disease; with more than 240 million chronically infected individuals, up to 600,000 will die from HBV-related illnesses annually.

HBV can survive for several days outside of the body and is commonly spread through sexual contact, sharing of contaminated needles and mother-to-infant transmission.

Acute symptoms of HBV include jaundice, loss of appetite, dark urine and pale stools; however, about half of those infected are asymptomatic.

Chronic carriers are at risk of liver damage (cirrhosis) and hepatocellular carcinoma with high morbidity and mortality.

With universal vaccination programs and improved hygiene, incidence rates have decreased since 1980s.

Areas that need to be addressed to properly combat HBV include vaccine hyporesponsiveness, inability to sustain seroprotective anti-HBs titers, more rapid seroprotection and reduced number of vaccine injections.

HBsAg is the main antigen of commercial vaccines because it comprises a highly immunogenic determinant. It is also an important marker for the risk of chronic HBV liver disease and cancer.

HEPLISAV™ is a vaccine comprising synthetic immunostimulatory sequences (1018 ISS) and Hepatitis B surface antigen (HBsAg).

Phase III trials demonstrated that HEPLISAV™ produces rapid, high titer and sustained seroprotection in healthy and hyporesponders with fewer immunizations and has been shown to have a similar safety profile to comparators.

Current HBV vaccines have shown to have a long-standing and excellent safety record; this can be seen as a significant hurdle to any future HBV vaccine development and licensure.

Current Treatment Options

Approved North American HBV vaccines. Currently, in Canada and the United States, there are only two commercially available vaccines that solely and specifically target HBV: Engerix-B® from GlaxoSmithKline Inc., and Recombivax HB® from Merck Sharpe and Dohme, a subsidiary of Merck and Co., Inc.

Engerix-B®. Engerix-B® is a recombinant hepatitis B vaccine, consisting of HBsAg expressed in the yeast *Saccharomyces cerevisiae*, which is adsorbed on aluminum hydroxide. The 24 kDa recombinant HBsAg, originating from an *adw* HBV subtype,[34] assembles into 20-nm particles when expressed by yeast; these particles have similar immunological and physical properties to the surface antigen that is isolated from human plasma during natural infection.[35,36] Engerix-B® was approved by the Food and Drug Administration (FDA) in 1989 as an intramuscular immunization. It contains no more than 5% yeast protein. The recommended dosage for neonates, infants, children and adolescents up to 19 y of age is 0.5 ml which contains 10 μg of HBsAg adsorbed onto 0.25 mg of aluminum hydroxide given three times at 0, 1 and 6 mo. For adults 20 y and over, the dosage is increased to 20 μg of HBsAg and 0.5 mg of aluminum hydroxide in a 1 ml volume with the same immunization schedule. Finally, for patients who are on hemodialysis, there are four immunizations at 0, 1, 2, and 6 mo in order to achieve a seroprotection rate of about 70%.[37,38] This population would receive a 2-ml dose for each vaccination, comprising 40 μg of HBsAg adsorbed on 1 mg of aluminum hydroxide.

The efficacy of Engerix-B® was made apparent from many long-term studies. In a 5-y review, where over 13,500 recipients received the vaccine, excellent results were observed across all ages. After the 3-dose regimen, there was 99 and 98% seroprotection in adults and children, with geometric mean anti-HBs titers (GMT) being 1085 and 4023 mIU/ml, respectively.[39] Another 3-dose regimen (0, 1 and 2 mo) study revealed rapid and high (96%) seroprotection adults; however, GMT anti-HBs titers after the third immunization were lower than the 0-, 1- and 6-mo schedule and is thus reserved as a schedule for inducing rapid protection. Also, an additional immunization at 12 mo for the 0-, 1-, and 2-mo schedule is recommended to ensure more robust and persistent anti-HBs titers.[39]

In a clinical trial performed in adolescents 11 to 15 y of age, it was found that the onset of seroprotective antibody anti-HBs titers was slower with the 2-dose schedule (0 and 6 mo) of Engerix-B® 20 μg (11.3% at month 2, 26.4% at month 6) compared with the 3-dose schedule (0, 1 and 6 mo) of Engerix-B® 10 μg (55.8% at month 2, 87.6% at month 6). It was observed that higher seroprotection rates were reached one month after the complete vaccination course with both studied schedules (96.7% with the 2-dose vs. 98.2% with the 3-dose schedule). However, geometric mean anti-HBs titers achieved in the study were 2,739 mIU/mL and 7,238 mIU/mL for 2-dose and 3-dose schedules respectively. This difference has implications in terms of the duration of seroprotection, since levels can rapidly decline one year after complete vaccination, despite anamnestic immune responses.

According to files from GlaxoSmithKline, Engerix-B® demonstrated other noteworthy results. Females generally seroconverted more quickly than males and anti-HBV titers were found to be higher in females than in males after 3 vaccine doses. The duration of seroprotection was also demonstrated when rates of 84.4% in the 2-dose group and 94.7% in the 3-dose group were found at 54 mo post-primary immunization. Other clinical studies with 20 μg and 10 μg dose for healthy adults and infants respectively also resulted in seroprotection rates in excess of 94%.

Clinical trials with Engerix-B® to assess safety profile of residual yeast-associated contaminants revealed no proven yeast hypersensitive reactions and no changes in specific anti-yeast antibodies.[36,40] Engerix-B® has been tolerated extremely well by all ages over the 25 y this vaccine has been available. The most common side effect was mild pain at the injection site.

Recombivax HB®. Recombivax HB®, licensed in the U.S. in 1986, also known as H-B-Vax® II in Europe and other countries outside of the U.S., is another non-infectious subunit viral vaccine utilizing HBsAg produced by recombinant DNA technology in yeast cells. Similar to Engerix-B®, HBsAg is expressed by its gene from the *adw* HBV subtype in yeast. Once harvested and purified (with less than 1% yeast protein), it is co-precipitated with aluminum sulfate adjuvant. The most common immunization schedules include three intramuscular injections at 0, 1 and 6 mo. Those up to 19 y of age are given a dose of 0.5 ml containing 5 μg of HBsAg and 0.25 mg of aluminum sulfate. Adults 20 y or more are given a 1.0 ml volume containing 10 μg of HBsAg and 0.5 mg of adjuvant. Dialysis patients are given 40 μg doses of HBsAg with the corresponding amount of aluminum sulfate.

Clinical studies have shown that intramuscular administrations of Recombivax HB® have been efficacious for many age groups. According to files from Merck Research Laboratories, seroprotection was achieved in 100% of infants, 99% in children and 99% in adolescents when given the 5 μg 3-dose regimen. Furthermore, at this dosage, studies suggested that this vaccine demonstrated a 95% efficacy in preventing chronic hepatitis B infection for infants born to mothers who were positive for HBeAg and HBsAg compared with untreated controls.[41] Merck also demonstrated 96% seroprotection in adults and 89% in adults over 40. In predialysis and dialysis patients, the 40 μg dose of Recombivax HB® resulted in 86% seroprotection after the completion of the immunization schedule. Interestingly, the route of immunization is critical for seroprotection; according to Merck, if administration was applied to the buttocks or a combination of buttocks and deltoid, seroprotection dropped to 55%. The drop in seroprotection rate following injection to buttocks has also been shown for injection to overweight subjects and for subcutaneous immunization; it is believed that injection into

adipose tissue rather than muscle tissue was the main factor for lower seroprotection.[42,43]

Clinical trials have shown that a single dose of HBV vaccines can adequately induce memory B-cells.[44] To demonstrate this, Recombivax HB® was given as 10 μg or 20 μg to two different groups as two doses, 6 mo apart. After the second dose, both groups showed 97–99% seroprotection. Two years later, 25% of the participants receiving 10 μg were no longer seroprotected (i.e., GMT < 10 mIU/ml). However, a booster immunization resulted in 100% seroprotection 3–4 weeks after the booster immunization.[45]

Like Engerix-B®, Recombivax HB® has been shown to be tolerated very well and induce very few side effects.[27] There was some concern about this vaccine after one adolescent developed multiple sclerosis (MS). However, additional studies failed to show an association between the vaccine and MS; in 1997, the WHO declared that the incident was not related to Recombivax HB®.[46]

To compare the two commercialized vaccines, a study involving adolescents was conducted. Each group of Engerix-B® (10 μg) and Recombivax HB® (5 μg) participants received three injections according to the recommended dose for adolescent immunization at 0, 1 and 6 mo.[47] The GMT reported by the Engerix-B® group was significantly higher (3691 mIU/ml) than the Recombivax HB® group (1001 mIU/ml). While there were no differences in gender or age in the groupings, the lower GMT values in the Recombivax HB® group are most likely due to the lower amount of antigen present in the vaccine. More importantly, however, 1 mo after the completion of vaccine regimen, there were no significant differences between the rates of seroprotection from both groups (98% Recombivax HB®, 99% Engerix-B®) or at any point that was tested for seroprotection during the vaccination schedule. There were also no significant differences in the reports of side effects from each group that were related to the study. The most common reports of systemic side effects were fatigue and headaches. There was one report of severe soreness from the site of injection from the Recombivax HB® group, but it was resolved by the end of the follow-up period.

Since infants receive many immunizations early in life and that the HBV vaccines do not interfere with the immune responses from other vaccines and vice versa, combination vaccines containing HBsAg exist in order to decrease the number of injections needed for routine vaccination. These include Comvax® (HBsAg from Recombivax-HB®, *Hemophilus influenza* type b) and Infanrix hexa® [combined diphtheria and tetanus toxoids, acellular pertussis (DTaP)], HBsAg from Engerix-B®, inactivated poliomyelitis, adsorbed conjugated *Hemophilus influenza* type b). There are also combination vaccines that can be given to travelers to endemic areas, such as Twinrix (inactivated hepatitis A virus, HBsAg from Engerix-B®). However, this article will focus on single-antigen vaccines.

Other commercially available global HBV vaccines. Many of the other HBV vaccines that have been approved around the world use similar amounts of HBsAg that have been expressed and purified from yeast. Also, most of them use alum as the adjuvant of choice due to its extensive safety record. It should be noted that most of these vaccines are interchangeably effective in promoting seroprotection against HBV.[3] For the sake of the awareness of other effective HBV vaccines, some of the more notable vaccines are discussed here.

Fendrix®. Fendrix®, another GlaxoSmithKline product, was approved for sale in Europe by the European Commission in 2005. Each 20 μg adult dose of recombinant HBsAg in Fendrix® is also produced by *S. cerevisiae* from the same source and percent purity as Engerix-B® However, Fendrix® is the first vaccine to be approved with a human adjuvant other than just alum. Fendrix® uses the adjuvant system ASO4, which consists of aluminum phosphate (0.5 mg) and monophosphoryl lipid A (MPL, 50 μg). MPL is derived from the lipopolysaccharide in Gram-negative bacteria and is one of the more potent stimulators of immune responses, more so than alum. Thus, due to the higher antibody anti-HBs titers induced by Fendrix®, its use is particularly targeted to those at high risk for HBV infections, such as dialysis patients, since they usually have lower immune responses compared with healthy individuals. Fendrix® is administered as four injections at 0, 1, 2, and 6 mo.[48] In one major study involving a single dose of Fendrix® (20 μg HBsAg) and a double dose of Engerix-B® (40 μg), by month 2, seroprotection rates induced by Fendrix® was 50% compared with 20% for Engerix-B® in dialysis patients. The seroprotection rates increased to 75% and 50% respectively by month 3. By month 7, even when seroprotection rates (~85–90%) were nearly similar between the two vaccines, GMT induced by Fendrix® was 3-fold greater than Engerix-B®.[49] Throughout the course of the 42-mo study, seroprotection rates in hemodialysis patients were consistently higher with Fendrix® recipients; at the end of the trial, seroprotection in this hyporesponsive population was still statistically higher with Fendrix® (78%) compared with Engerix-B® (52%). With respect to side effects, there were significantly more reports of site injection pain using Fendrix® (41% vs. 19% Engerix-B®); however, all of the events resolved within a 4-d period. There were no differences in the number of systemic adverse events between the two vaccines.

In other Phase II and Phase III clinical trials involving healthy adults, Fendrix® was consistently more effective in inducing 100% seroprotection with only two immunizations instead of three immunizations compared with Engerix-B®.[50-52] Moreover, after just 1 mo during the 6-mo immunization schedules, three independent studies showed that seroprotection rates and GMTs were 2- to 3-fold higher in individuals receiving Fendrix® compared with Engerix-B®.[50-52]

Hepavax-Gene®. Manufactured by Crucell (formerly Berna Biotech Korea Corp.), each 1-ml dose of Hepavax-Gene® contains 20 μg of HBsAg adsorbed onto approximately 0.5 mg of aluminum hydroxide. The HBsAg protein antigen in Hepavax-Gene® vaccine is expressed by the methylotropic yeast *Hansenula polymorpha*. On a 0-, 1-, and 6-mo regimen, seroprotection in healthy adults was similar to Engerix-B® (99% and 100%, respectively). However, on a 0-, 1- and 2-mo schedule, Hepavax-Gene® was found to induce superior seroprotection to Engerix-B® (94.2% and 86.4%, respectively).[53] In infants born to HBsAg and HBeAg-positive mothers, Hepavax-Gene® was found to be as effective as Engerix-B® in seroconversion; only 2% of infants

given Hepavax-Gene® and 3% given Engerix-B® became HBsAg positive after 2 y of the study.[54] Any adverse events reported did not differ from Engerix-B® recipients; the most common side effects included mild redness, soreness and swelling at the injection site. These usually subsided within couple of days post-vaccination.

Bio-Hep-B®. Manufactured by Bio-Technology General® Ltd. for sale in Israel, Bio-Hep-B® is currently the only vaccine where multiple HBV surface antigens are produced by cultivation in Chinese hamster ovary (CHO) cells instead of yeast. It consists of 22-nm particles isolated and purified from culture medium. The particles contain all three epitopes of hepatitis B surface antigen (HBsAg), namely S, pre-S1 and pre-S2, in their glycosylated and non-glycosylated forms, embedded in a phospholipid matrix, thus resembling the authentic plasma HBsAg. The adult dose of 10 μg of antigen is formulated by adsorption onto aluminum hydroxide in a final volume of 1 ml. The final preparation is virtually free of DNA and contains less than 3% protein contaminants. Clinical trials have shown in comparison to Engerix-B®, Bio-Hep-B® induced more seroprotection (85% and 98%, respectively) after the three dose regimen at 0, 1, and 6 mo.[55] Interestingly, the rapid onset of antibody responses induced by Bio-Hep-B® showed 66.5% seroconversion compared with just 19.3% by Engerix-B® in sera 1 mo after the first immunization, reflecting immunopotency of Bio-Hep-B® over Engerix-B®, in terms of dose sparing (10 μg and 20 μg HBsAg, respectively).[56] In neonates, these differences are even more pronounced; after just one immunization, infants receiving Bio-Hep-B® had 54% seroprotection, while Engerix-B® recipients only demonstrated 7% seroprotection.[57] In all clinical trials involving Bio-Hep-B® and Engerix-B®, both vaccines demonstrated safety and are well tolerated.

The Product: HEPLISAV™

Though alum is the most widely used adjuvant in humans, due to its extensive safety record, it has a strong bias toward Th2 antibody-mediated responses; it induces minimal Th1 cell-mediated immunity and often requires multiple booster immunizations.[58-60] Most HBV commercial vaccines use yeast-derived recombinant HBsAg adsorbed to alum (aluminum hydroxide or aluminum phosphate) as adjuvants. Most current vaccines are limited in that (1) immune responses are reduced in adults 40 y of age and older; (2) percent seroprotection is also reduced in dialysis patients, smokers, or the obese; (3) the time to achieve seroprotection is prolonged (e.g., 6–12 mo);[28] and (4) seroprotection requires compliance to a 3- or 4-dose injection regimen. The latter two points are particularly problematic for individuals who are in high-risk environments. This includes health care workers, travelers, patients on dialysis, injected drug users or those who may be less compliant to follow a prolonged immunization regimen such as adolescents. While different immunization schedules are available, such as three immunizations in three weeks, or 2-dose schedules, optimal seroprotection is not achieved, and ultimately, another booster administration is still required 4–6 mo post primary immunization.[27]

Dynavax Technologies has been developing a very promising HBV vaccine candidate that has completed Phase III clinical trials.[61,62] Recombinant HBsAg is expressed in the methylotrophic yeast *H. polymorpha*.[63] Briefly, this yeast strain is stably transformed with expression vectors that are under the control of promoters from methanol oxidase (*MOX*) and formate dehydrogenase (*FMD*). Upon induction of HBsAg expression with methanol, the yeast is harvested and homogenized with glass beads where the supernatant is collected and purified through a series of steps that include cesium chloride density gradient separation, size-exclusion chromatography and sterile filtration.[63] HBsAg purity is > 95%, which is the standard limit as recommended by the WHO. Other impurities, according to U.S. Patent 6428984, were well under the WHO and European Pharmacopoeia recommended limits, including residual DNA content (< 10 pg/dose, where dose is 20 μg of HBsAg), cesium content (< 10 μg/dose) and endotoxin levels (< 100 endotoxin units). The key difference in this vaccine is the use of synthetic immunostimulatory sequences (ISS) as adjuvants instead of alum currently used in marketed vaccines. ISS are cytosine phosphoguanosine (CpG) motifs that have bacterial DNA origin and have stimulatory effects on the immune system.[64] The CpG motifs stimulate the innate immune system through Toll-like receptor-9 (TLR-9), which results in efficacious immunological effects, such as the production of IL-12, IL-18, and IFN-γ from macrophages and natural killer (NK) cells, promoting Th1 responses for both protein and DNA-derived vaccines.[65-67] They have also been shown to be involved with antibody production and B cell proliferation.[68-70] The 1018 ISS from Dynavax is a 22-mer, 7.15 kDa phosphorothioate oligodeoxyribonucleotide (sequence 5'-TGACTGTGAA CGTTCGAGAT GA-3') that has shown to be active in vitro and in vivo in many species, including humans.[71]

Preclinical studies. In addition to a variety of animal species (mice, rabbits and dogs), 1018 ISS was also shown to enhance protective immune response against HBV in non-human primates (e.g., baboons and monkeys).[72] Other primate studies showed that 80% of immunized primates demonstrated seroprotection after single immunization of HBsAg and 1018 ISS, compared with only 20% with those immunized without 1018 ISS. This was followed by another study showing that despite achieving seroprotection after one immunization, a third injection resulted in 3- to 50-fold higher antibody-specific anti-HBs titers compared with those induced with HBsAg alone.[73] Finally, anti-HBs titers were on average, nearly 45-fold greater than the same administration of HBsAg, but with alum as the adjuvant.[73] These results provided an excellent basis for support into clinical trials.

Phase I studies. The first Phase I study examined the efficacy of various doses of 1018 ISS and their safety profiles. In this study, all products were stored at < −60°C and used within 8 h of thawing. The formulation was diluted in phosphate-buffered saline until the desire concentrations were achieved. Formulations included 20 μg of recombinant HBsAg and one of 300, 650, 1000, or 3000 μg of 1018 ISS.[74] Forty-eight individuals, aged 18–55, were recruited to the study and were placed into four study groups of 12 each where each dosage of 1018 ISS was

examined. In a 2:2:8 ratio, participants received HBsAg, 1018 ISS, or the combination of the two, respectively. Two doses of each vaccine were given 2 mo apart.

The vaccine was tolerated in all groups except for one participant from the HBsAg + 1,000 μg 1018 ISS group, who developed swelling, muscle pain, shortness of breath, dizziness, fatigue and erythema after only one immunization. The most commonly reported injection site adverse events such as pain and tenderness, increased as the dosage of ISS increased. The second immunization did not increase the number of adverse events. All symptoms were resolved without medical intervention within 24 h except myalgia, which resolved in 3 d post immunization.

Most individuals (14/16) who received the HBsAg and 1,000 or 3,000 μg of 1018 ISS demonstrated seroprotection after 1 mo post-initial immunization. This proportion became 100% for individuals receiving 650, 1,000 or 3,000 μg of ISS as soon as one week after the final booster immunization (9 weeks post-initial immunization) where geometric mean anti-HBs titers (GMT) were 82, 316 and 1,429 mIU/ml, respectively ($p < 0.001$ compared with ISS or HBsAg alone). GMT further increased 4 weeks after the second immunization where anti-HBs titers were 878, 1,545 and 3,045 mIU/ml ($p < 0.001$ compared with ISS or HBsAg alone). Furthermore, 100% seroprotection in these participants persisted 4 mo after the booster immunization. Participants who only received HBsAg did not achieve seroprotective anti-HBs titers at any point during the study. Since 3 mg of 1018 ISS was found to be most effective dose in enhancing the magnitude of the immune responses specifically against HBV, this adjuvant dosage (molar ratio 500:1, ISS:HBsAg) has been used in all subsequent trials.

Another Phase I study investigated whether the amount of time in between HEPLISAV™ immunizations could be reduced, since high-risk groups such as health care workers would benefit from more rapid seroprotection.[61] Forty-one participants were enrolled in this study, where 18 were given an immunization regimen of 0 and 4 weeks, while 23 were schedule for 0 and 8 weeks. As early as 8 weeks, the 0–4 weeks study group (4 weeks after second injection) had 94% seroprotection (GMT 244 mIU/ml), while the 0–8 weeks study group exhibited 70% seroprotection (from only one injection, GMT 16 mIU/ml, $p < 0.001$). At 12 weeks, both groups had achieved seroprotection; this percentage was maintained for the balance of the 32-week study where GMT anti-HBs titers were still at 439 and 864 mIU/ml ($p = 0.038$) for the 0–4 week and 0–8 week study group, respectively. These results suggest that a more rapid immunization schedule could effectively provide early seroprotection levels, thereby possibly improving compliance and convenience. Adverse events (pain at injection site, myalgia, malaise and fatigue) were reported with similar frequency regardless of immunization schedule; no serious adverse events were reported.

Phase II studies. Two Phase II clinical trials further exemplified HEPLISAV™'s superiority in providing effective HBV seroprotection. Each study evaluated the vaccine in different age groups: 18- to 28-y-old adults[75] and adults aged 40–70 y.[76]

The former was an observer-blind study of 99 participants; 51 were to receive Engerix-B (20 μg of HBsAg adsorbed to 0.5 mg of aluminum hydroxide adjuvant in 1 ml dose) vaccinations at 0, 8 and 24 weeks while 48 received two doses of HEPLISAV™ (20 μg of HBsAg mixed to 3 mg of 1018 ISS in 0.5 ml dose) at 0 and 8 weeks, while receiving a third dose at 24 weeks of a quadrivalent meningococcal vaccine to maintain the observer-blindness. Four weeks after the priming dose, 79% of HEPLISAV™ and 12% of Engerix-B® recipients achieved seroprotection (GMT 23 and 2 mIU/ml, respectively, $p < 0.001$). One week after the second immunization, protective levels were 100% (GMT 1,603 mIU/ml) and 18% (GMT 2 mIU/ml), respectively ($p < 0.001$ between GMT values), demonstrating the superior magnitude of antibody responses elicited by HEPLISAV™. Four weeks post-second immunization, participants receiving HEPLISAV™ were still fully seroprotected with GMT of 2,074 mIU/ml, while the other group receiving Engerix-B® showed 64% protection at 32 mIU/ml GMT ($p < 0.001$ between GMT values). Engerix-B® recipients did not achieve similar seroprotection rates until one month after the third dose at 24 weeks, while those who received HEPLISAV™ maintained 100% seroprotection without a third dose. After 1 y post-second immunization, seroprotection remained at 100% for HEPLISAV™ and 89.6% for Engerix-B® recipients. These results for HEPLISAV™ were encouraging since HBV seroprotection could be achieved with one less dose, and the time required for seroprotection reduced from 7 to 2 mo, while maintaining a similar safety profile as Engerix-B®.

In the same study, while there were increased reports of mild injection pain for recipients of HEPLISAV™, the rates of adverse events did not differ after each immunization. Also, the number of systemic adverse events between the vaccine groups was found to be insignificant; no serious adverse events related to the study vaccines were reported.

Of particular interest is the study of the older adult group (40–70 y), since they are often more immuno-hyporesponsive than younger adults. This group included 409 eligible, exclusively Asian participants; overweight individuals and smokers were also included. Two principal groups received three doses of HEPLISAV™ or Engerix-B®. However, the regimen for HEPLISAV™ was 0, 2, and 6 mo, while Engerix-B® was 0, 1, and 6 mo, somewhat limiting a proper comparison of their activities. Regardless, those receiving HEPLISAV™ showed a much higher magnitude of antibody anti-HBs titers compared with Engerix-B® users after 12 weeks (GMT 256 mIU/ml and 6 mIU/ml, $p < 0.001$) post-priming injection while demonstrating seroprotection rates of 96.6% and 24%, respectively. Four weeks after the final immunization (28 weeks), protective anti-HBs titers were 100% in participants receiving HEPLISAV™ (GMT 1698 mIU/ml) compared with 73.1% in patients receiving Engerix-B® (GMT 569 mIU/ml). After 50 weeks of the study, seroprotection was maintained at 100% in HEPLISAV™ recipients, while reduced to 68.6% for participants receiving Engerix-B®. When divided into 40–55 and 56–70 age groups, complete seroprotection was maintained across both groups with HEPLISAV™, while Engerix-B® recipients demonstrated rates of 73 and 51%, respectively. Essentially, while 96.6% seroprotection was achieved 4 weeks post-secondary immunization

(28 weeks), such levels were not attained throughout the three Engerix-B® immunizations (50 weeks), providing strong evidence that HEPLISAV™ can achieve more rapid seroprotection with fewer immunizations than currently available vaccines, even in a less immune-responsive population such as older adults.

In the above study, the HEPLISAV™ group reported more mild/moderate injection site-pain after the second immunization than Engerix-B® (23.4% vs. 13%, respectively). One severe case on injection-site related pain was reported for Engerix-B® but was resolved within a day. Redness and swelling did not differ significantly between all injections for both groups. Systemic reactions such as headaches, fatigue and malaise were generally similar between each group except for the second injection, where more reports were recorded with Engerix-B® recipients (21%) than HEPLISAV™ (13.9%). Overall, most of the adverse effects were reported after the second immunization compared with the priming injection, regardless of the vaccine group.

In another Phase II study, in an attempt to facilitate rapid seroprotection in hemodialysis patients in the 40- to 70-y age group, studies showed that 100% seroprotection was achieved 20 weeks after two immunizations of HEPLISAV™, regardless of a regular (20 μg HBsAg; 3 mg 1018 ISS) or double (40 μg HBsAg; 6 mg 1018 ISS) dose of HEPLISAV™.[77] Each dose was given at 0, 4, and 24 weeks. However, this study was voluntarily discontinued before the third immunization due to a concurrent HEPLISAV™ study where a case of possible autoimmune response occurred.

Phase III studies. A Phase III trial testing the safety and immunogenicity of HEPLISAV™ in two relatively rapid doses (0 and 1 mo, with a placebo saline injection at 6 mo) was compared with the standard regimen of three Engerix-B® vaccinations (0, 1 and 6 mo).[62] This multi-center, observer-blinded study randomized 2,415 patients in a 3:1 ratio of HEPLISAV™ and Engerix-B® recipients, respectively. At all time points during this study, seroprotection by HEPLISAV™ was statistically superior to Engerix-B®. At 8 weeks, seroprotection was 88.5% and 26.4%, respectively; at 24 weeks, 98.3 and 32.4%, and at 28 weeks, 97.9 and 81.1%. Upon completion of both injection schedules, seroprotection after two doses of HEPLISAV™ was 95% (12 weeks post-priming immunization) while the three doses of Engerix-B® resulted in 81% seroprotection (28 weeks post immunization). Differences in seroprotection was even greater in older adults (age 40–55), where the rates were 92% and 75%, respectively, after both HEPLISAV™ and Engerix-B® participants received the complete scheduled immunization dosage. However, by the conclusion of the study (28 weeks), the GMT was higher in younger adults (age 18–39) than the older adults (age 40–55) by 2-fold in HEPLISAV™ and 3-fold in Engerix-B® recipients. It was only at week 28 of the study that seroprotection rates elicited by Engerix-B® (4 weeks after last immunization) were statistically similar to HEPLISAV™ (24 weeks after last immunization), even though seroprotection by the latter was achieved as early as 8 weeks. While analysis of long-term protective effects (e.g., 1-y study) would have been ideal to evaluate the efficacy of both vaccines, these results strongly suggest that HEPLISAV™ may be ideal for not only the general population who need HBV vaccination, but particularly for the hyporesponsive populations that thus far, have been unable to achieve seroprotection rates similar to younger, healthy adults.

Generally, both vaccines were well tolerated by the participants. There were more reports of pain at the injection site after the first injection of HEPLISAV™ (39%) compared with Engerix-B® (34%); however, these reports progressively declined in number after subsequent immunizations. Other local adverse events such as swelling and erythema were low in frequency (less than 5%). There were no other significant differences in systemic adverse effects such as headache, malaise or fatigue between vaccine groups or between HEPLISAV™ and saline placebo injection.

Since sample numbers are often relatively small in clinical trials, it is possible that any vaccine candidate, including HEPLISAV™, may induce autoimmune responses.[73] Interestingly, an individual was excused from the study after developing Guillain-Barré syndrome 110 d after the second HEPLISAV™ dose.[62] However, it was discovered that the same individual also had received an influenza vaccine 5 d prior to the diagnosis of Guillain-Barré syndrome. With medication, there was improvement in the patient's condition. It was determined by the investigator that while severe, this case was not related to the study, but from the influenza vaccine; the subject did not continue in the study. Another participant who received two doses of HEPLISAV™ developed inflammation of the blood vessels (Wegener's granulomatosis), and one recipient who received Engerix-B® also experienced systemic vasculitis after the second dose.[62] The single case of granulomatosis with the HEPLISAV™ group resulted in the early termination of the aforementioned Phase II trial comparing single and double dose of HEPLISAV™.[77] Upon further review of the autoimmune markers studied in the Phase III trial [antinuclear antibodies (ANA) and anti-double-stranded DNA (anti-ds-DNA)], there were no significant changes of ANA or anti-ds-DNA between recipients who received either vaccine. Consequently, the U.S. Food and Drug Administration permitted further development of HEPLISAV™. A summary of these clinical trials are summarized in **Table 1**. An overview of the ability of HEPLISAV™ to induce seroprotection as a function of time is summarized in **Table 2**.

Commercial and Public Health Issues

Since 1018 ISS is a TLR-9 agonist, Coley Pharmaceutical Group challenged Dynavax in its development of HEPLISAV™, as Coley has a patent estate on TLR-9 technology. However, in 2007, the two companies reached a licensing agreement with Dynavax being able to commercialize and market HEPLISAV™ as a prophylactic hepatitis B vaccine through a non-exclusive license under Coley's immunostimulatory oligonucleotide patent estate. In return, Coley received a $5 million up-front payment, a potential for up to another $5 million once HEPLISAV™ acquires regulatory approval and royalty payments from future sales of HEPLISAV™.

Development of HEPLISAV™ was halted by the FDA from the one incidence each of Wegener's granulomatosis and

Table 1. Summary of HEPLISAV clinical trials

Phase	Description	Dosage/regimen	Results	Safety profile	Reference
I	• 48 healthy adults aged 18–55 with no prior exposure to HBV or vaccines and had negative tests for HBsAg and its antibodies	• 4 equal groups of 12 • in one group, in a 2:2:8 ratio, individuals given 20 μg of HBsAg, 300 μg of ISS or 20 μg of HBsAg + 300 μg of HBsAg, respectively • other three groups received similar formulations except for increasing ISS amounts (650, 1000 and 3000 μg) • 2 doses, 0 and 2 mo	• 100% seroprotection in groups receiving HBsAg + 650, 1000 or 3000 μg ISS as soon as 7 d after second injection (9 weeks after priming injection)	• mild tenderness at injection site more frequently reported with HBsAg + 1,000 or 3,000 μg ISS • injection site adverse events did not increase with second injection • motion pain with HBsAg + 3,000 μg group • all short and self-limiting duration	74
I	• 41 healthy adults aged 18–39	• 2 groups that are given 2 doses on 2 different schedules; each receiving either HBsAg + 3 mg ISS at 0 and 4 weeks, or 0 and 8 weeks	• 100% seroprotection in both groups as soon as 12 weeks after priming injection	• no significant differences in reports of adverse events between the two groups	61
II	• 99 healthy adults aged 18–28 to compare HEPLISAV with comparator Engerix-B, observer-blinded	• 2 random groups given HEPLISAV (2 doses given 0 and 8 weeks) or Engerix-B (3 doses given at 0, 8 and 24 weeks)	• 100% seroprotection in HEPLISAV recipients after 9 weeks post priming injection, 18% with Engerix-B recipients • equivalent seroprotection by Engerix-B not achieved until 28 weeks after first injection • fewer HEPLISAV injections need to get more rapid seroprotection	• no serious adverse effects deemed as related to the study vaccines • more mild injection site pain associated with HEPLISAV • no differences in systemic adverse events between groups	75
II	• 409 healthy adults aged 40–70 from Korea, Philippines, Singapore to compare HEPLISAV and Engerix-B	• 2 random groups who receive three injections of either HEPLISAV (0, 8 and 24 weeks) or Engerix-B (0, 4 and 24 weeks) • placebo injection given at 4 weeks for HEPLISAV recipients, 8 weeks for Engerix-B	• seroprotection at 96.6% at 12 weeks after first HEPLISAV injection, 24% seroprotection for Engerix-B users • at 28 weeks, seroprotection from HEPLISAV and Engerix-B users are significantly different (100% vs. 73.1%, respectively)	• injection-site pain more prevalent after second HEPLISAV injection compared with Engerix-B • systemic adverse effects (headache) reported more after second Engerix-B injection compared with HEPLISAV • serious adverse events were not deemed related to study vaccines	76
II	• 41 participants aged 40–70 y with end-stage renal disease, 15 were on dialysis	• 2 groups were given three injections of HEPLISAV at 0, 4 and 24 weeks • each group given a single dose (20 μg HBsAg, 3 mg ISS) or a double dose (40 μg HBsAg, 6 mg ISS)	• at 24 weeks, after the first injection, 100% seroprotection in both groups • at 12 weeks, double dose recipients showed higher seroprotection than single dose (74 vs. 43%) • seroprotection can also be achieved in hyporesponsive populations	•single dose had 3- to 5-fold fewer general reactions compared with the double dose	77

Guillain-Barré syndrome;[62] however, the hold was lifted since in the former case, there were no differences in autoimmune markers between HEPLISAV™ and the commercially-available Engerix-B®, and that in the latter, the investigator determined that the syndrome development was not related to the study. Seven other reported events from the application of HEPLISAV™ that were deemed as potentially autoimmune included hypothyroidism, Bell's palsy, erythema nodosum and vitiligo. Despite the efficacy of HEPLISAV™, an FDA Advisory Committee determined that there was insufficient data to support its safety record. Factors such as the limited follow-up periods, that the safety profile of 1018 ISS is limited only to the HEPLISAV™ product and that this product has potential to cause autoimmunity were some of the reasons given by the FDA. It was also determined that the pre-licensure safety database for HEPLISAV™ may not be sufficient to detect rare adverse events for the sample size from the Phase III trial.[78] As such, recommendations include post-marketing evaluation of HEPLISAV™ in a larger sample population for a longer duration of study (e.g., 1 y). Indeed, in late February 2013, a Complete Response Letter from the FDA stated that Dynavax's application

Table 1. Summary of HEPLISAV clinical trials (continued)

Phase	Description	Dosage/regimen	Results	Safety profile	Reference
III	• 2415 healthy adults aged 18–55	• randomized into two groups in a 3:1 ratio (HEPLISAV:Engerix-B) • age stratification of 11–39 and 40–55 • HEPLISAV recipients given two injections at 0 and 4 weeks • Engerix-B recipients given three injections at 0, 4 and 24 weeks	• at 8 weeks after the initial injections, seroprotection for HEPLISAV recipients was 88.5% vs. 26.4% for Engerix-B users • at 24 weeks, seroprotection rates were 98.3 and 32.4%, respectively •at 28 weeks, 4 weeks after the third injection of Engerix-B, seroprotection rates were 97.9 vs. 81.1%, respectively • in the older adult group, after completion of the entire scheduled injections, seroprotection was still higher in HEPLISAV recipients (92% after 12 weeks) compared with Engerix-B (75% after 28 weeks)	• both vaccines tolerated well • slightly more reports of injection site pains in HEPLISAV group than Engerix-B (39 vs. 34%), less reports with subsequent injections for both vaccines • no differences in systemic adverse events between groups • one incidence of Wegener's granulomatosis and Guillai-Barre syndrome in the HEPLISAV group, deemed unrelated to the vaccine • 7 possible reports of autoimmunity include hypothyroidism, Bell's palsy, erythema nodosum, vitiligo	62

Table 2. Summary of seroprotection rates in HEPLISAV clinical trials

Clinical phase (description of clinical trial)	Seroprotection rate (%) (≥10 mIU/ml)							
	4 weeks	8 weeks	9 weeks	12 weeks	20 weeks	24 weeks	28 weeks	≥ 30 weeks
I (ISS dose range study)		38* (650 μg)	100**	100**			100**	
		100* (1,000 μg)	100**	100**			100**	
		87* (3,000 μg)	100**	100**			100**	
I (time between immunizations with 3 mg ISS)		94** (0–4 weeks)		100**				100**
		70* (0–8 weeks)		100**				100**
II (ages 18–28 y)	79* (ISS)	75*	100**	100**			100**	100**
	12* (Eng)	4*	18**	64**			≥90***	≥95***
II (ages 40–70 y)	14* (ISS)	24**		97**			100***	100***
	3* (Eng)						73***	69***
II (single vs. double dose)					100** (single)			
					100** (double)			
III (two ISS doses vs. three Eng doses)	24* (ISS)	89**	95**			98**	98**	
	4 *(Eng)	26**				32**	81***	

ISS, HEPLISAV; Eng, Engerix-B; number of asterisks indicate number of immunizations that induced seroprotection.

for the use of HEPLISAV™ for the 18–70 age group would not be approved without additional safety evaluations. Since CpG adjuvants have yet to be approved for human vaccines, there are still concerns about the possibility of the induction of rare autoimmune events. It is possible that Dynavax may eventually conduct additional safety studies; currently, however, the company has indicated their desire to work with the FDA to get HEPLISAV™ approved for more focused populations, such as older adults aged 40–70, or individuals with chronic kidney disease, who would stand to benefit more from HEPLISAV™. The FDA has also requested additional information pertaining to quality control on manufacturing and validation of HEPLISAV™ production. Another Phase III study (NCT01005407) to demonstrate lot-to-lot consistency among individuals aged 40–70 is currently ongoing.

Conclusions

The mechanism of 1018 ISS and how it exerts its adjuvant effect with HBsAg is not well described. Some studies have shown that when CpG is added to HBsAg and alum, it is possible that the specific antibody responses against HBsAg are a result of enhanced late affinity maturation.[79] Indeed, clinical studies have shown that addition of CpG 7909 to Engerix-B® to healthy adults induced seroprotection and enhanced cytotoxic T-cell immune responses as soon as two weeks after the priming immunization, despite reports of more mild-moderate side effects.[80] When CpG and Engerix-B® was given to HIV patients, there was enhanced seroprotection (100%) 10 mo after the final immunization compared with recipients who received only

Engerix-B® (63%).[81] While 1018 ISS likely mediates its adjuvant effect through TLR-9, it will be important to determine how it does this with HBsAg in the future. Nonetheless, these results support the future use of CpG in human vaccine formulations, after extensive safety studies due to its potency. Upon approval, HEPLISAV™ would represent the first licensure of a CpG adjuvant, as a novel class of adjuvants for human vaccines and immunotherapeutics.

Current vaccine strategies that use HBsAg as the antigen in the formulations, including HEPLISAV™, may have some disadvantages. HBsAg from HBV is prone to random mutations, and thus constant monitoring is required to ensure that current vaccines remain effective. One mutant, G145R, abrogated the immunogenic "a" antigenic determinant and impaired surface antigen secretion.[82-84] As a result, this mutant escaped B cell immune responses and resulted in infection. Interestingly, chimpanzee studies demonstrated that the HBV vaccines licensed in the US were able to confer broad protection in vivo against the G145R mutant.[85] However, this may not be the case with other mutants and those in the future; "vaccine escape mutants" have also been described at the T-cell level.[86] As a result, while it may not be necessary to change vaccine formulations or strategies for every mutant that is discovered, it is imperative to continue epidemiological surveillance for HBV mutants to ensure the efficacy of the current vaccines.

A vaccine that is able to elicit seroprotection against HBV with fewer immunizations and in more rapid succession, particularly among immunologically hyporesponsive populations is ideal to reduce the morbidity and mortality of chronic HBV infections effectively. The use of 1018 ISS in HEPLISAV™ as an adjuvant and with its inherent immunological Th1 properties may have satisfied these needs. As such, HEPLISAV™ has shown great promise in terms of immunogenicity, and has shown to be as safe as current vaccines on the market. HEPLISAV™ may trigger a new generation of human vaccines that do not use alum as the traditional adjuvant. It will be important to closely monitor individuals who receive HEPLISAV™ over a period of time to ensure that any autoimmune responses/adverse events are monitored and managed safely, particularly since 1018 ISS does not have the safety track record that alum does. The dosage of 1018 ISS in the majority of clinical trials was 3 mg. It would be interesting to examine whether a reduced dose of adjuvant such as 1 mg, which still showed great potency in Phase I trials, could mitigate the adverse events that have been reported so far. Given that the FDA has not approved HEPLISAV™ without further safety evaluation due to their concerns for autoimmune events, this is perhaps one option that may be considered. Certainly, at this point in its development and the costs associated with additional studies as a New Product in its own right, this option may be difficult to explore. Other methods of vaccine targeting should also be explored in hopes of generating specific immune responses where they are needed the most.

Notwithstanding, the decision of the FDA should not be a deterrent for future development of HBV vaccines, in particular, the use of novel but effective adjuvants. Adjuvants such as 1018 ISS are absolutely needed in the search for improved vaccine formulations. Since the inclusion of 1018 ISS enabled a reduction in the number of immunizations and provided more rapid seroprotection (**Table 2**), they are two great reasons why HEPLISAV™ should be a strong candidate for licensure; however, efficacy and safety are both important and future clinical trials need to be designed with this in mind, a major hurdle considering that there are already approved vaccines with strong safety records.

Disclosure of Potential Conflicts of Interest

FDM was an author of reference 62.

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